REMARKS

Claims 1-19 are pending in this application. Applicants have hereinabove amended claims 1, 2, 4, 5, 8-9, and 17-19 and added new claim 20. Thus, claims 1-20 are currently pending in the subject application.

Sequence Listing

Applicant submits as **Exhibit D** a substitute paper copy of the nucleotide and/or amino acid sequences disclosed in the application in order to revise the numbering, but not the substance of the sequences.

Applicant also submits herewith the formatted Sequence Listing in computer readable form which complies with the requirements of 37 C.F.R. §1.824 and correspond to the paper copy. In addition, applicants submit a Statement in Accordance with 37 C.F.R. §1.821(f), attached hereto as **Exhibit E**, certifying that the computer readable form containing the nucleic acid and/or amino acid sequences as required by 37 C.F.R. §1.821(e) contains the same information which is resubmitted as "Sequence Listing".

Rejection under 35 U.S.C. § 112, first paragraph

On pages 2-5 of the April 10, 2001 Office Action, the Examiner rejected claims 1-17 under 35 U.S.C. 112, first paragraph, as allegedly containing subject matter wich was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Examiner stated that the claims are broadly drawn to typing alleles of the minor histocompatibility antigen HA-1 comprising detecting polymorphic nucleotides in the cDNA or genomic nucleic acids of the alleles. However, the Examiner alleged that the

specification does not provide sufficient written description as to the sequence of the HA-1 antigen, or the cDNA or genomic DNA that encodes the full HA-1 antigen.

The Examiner acknowledged that the specification teaches allele typing of the HA-1 peptide which is disclosed as SEQ ID NO 17. Two alleles are present resulting from a sequence change at nucleotide position 8 of SEQ ID NO 17 (nucleic acid sequence that encodes the HA-1 peptide), the "R" allele (SEQ ID NO 17) and the "H" allele (SEQ ID NO 19) corresponding to an Arginine or a Histidine at the 3rd position of the HA-1 nonapeptide (VLXDDLLEA, where X is either arginine or histidine). The Examiner also acknowledged that the specification teaches that typing these two alleles is important in typing potential donors for bone marrow transplants to prevent Graft versus Host Disease (GVHD), as patients, from two families, receiving bone marrow transplants from HLA identical donors within family were found to develop GVDH; and that allele typing of the HA-1 nonapeptide showed that donors and recipients differed in the HA-1 allele (p 21, example 1). The Examiner also acknowledged that the specification fully teaches the skilled artisan how to type the "H" or the "R" allele in a subject and teaches the sequence of the HA-1 peptide (SEQ ID NO 17 or 19) (see figure 5, p. 5-6); and further teaches that the HA-1 peptide is encoded by 2 exons from the KIAA0223 gene (p 6 and 7), and teaches the sequence of the intron located between these two exons (SEQ ID NO 1).

However, the Examiner alleged that the specification does not teach the full sequence of the HA-1 *antigen*, nor does the specification teach the cDNA or genomic DNA that corresponds to the nucleic acid sequences that encode the antigen; that while the specification teaches that the KIAA0223 gene encodes the HA-1 antigen, it allegedly does not disclose what sequences within the

KIAA0223 gene correspond to the HA-1 gene. The Examiner alleged that it cannot be determined from the disclosure in the specification if the gene product of the KIAA0223 gene is the HA-1 *antigen*, wherein the HA-1 peptide is a peptide located withing the HA-1 antigen (The specification allegedly does not teach that the KIAA0223 gene is the HA-1 gene) or whether the complete sequence of the HA-1 antigen is the HA-1 nonapeptide (SEQ ID NOS 17 OR 19) as the specification states that The GvHD associated mH antigen HA-1 is a nonapeptide derived from the di allelic KIAA0223 gene (p. 21). The Examiner alleged that as the claims are drawn to typing allegedly unidentified alleles in undisclosed sequences, and the specification allegedly does not adequately describe the breadth of these undisclosed sequences, each of the claimed inventions is a genus for which a representative number of species for each genus must be disclosed to meet the written description requirement of 112, first paragraph, referencing to *Vas Cath Inc. V. Mahurkar*, 19 USPQ2d 1111, (the written description must convey to one of skill in the art "with reasonable clarity" that as of the filing date applicant was in possession of the claimed invention). The Examiner alleged that absent a written description disclosing the full sequence of the HA-1 antigen (if the HA-1 peptide does not represent the full sequence of the HA-1 antigen) or the sequence of the KIAA0223 gene that correspond to the cDNA or genomic sequences that encode the HA-1 antigen (if the HA-1 peptide does not represent the full sequence of the HA-1 antigen), the specification fails to show that applicant was, in fact, "in possession of the claimed invention" at the time the application for patent was filed.

With regard to claim 13, the Examiner alleged that the claim is broadly drawn to an isolated nucleic acid displaying "80% sequence homology" to SEQ ID NO 1, 17 or 18 or any fragment that can be used for HA-1 typing, and that many sequences are

encompassed by applicant's claims, and particularly those having "80% sequence homology" or any fragment of such would bear little resemblance to the single HA-1 peptide (VLXDDLLEA) and intronic sequence (SEQ ID NO 1) taught in the specification. The Examiner alleged that neither the claims nor the specification set forth any structural or functional characteristics that a skilled artisan could use to identify polynucleotides such polynucleotides other than by SEQ ID NO 1.

In response, applicant respectfully submits that claimed invention of claims 1-10 and 14-17 is a method (or a kit) for typing of alleles. For these claims, it should only be required that the inventor shows that at the moment of the invention, the inventor was able to discriminate between alleles. This is exactly what it shown in the subject specification. The inventors have found differences that can be detected in a way which provides information is about the type of allele(s) a particular subject contains.

Claims 11-14 were also in possession of the inventors at the time the application was filed. Claims 11-14 are concerned with nucleic acids (primer, probes or isolated) that can be found with the information given in the application. The application discloses several probes, primers and isolated nucleic acids that fulfil the criteria recited in the claims. The inventor should not be required to exhaustively list all possible variations. Rather, the inventor need merely to have indicated how such variants can be made. Indeed, the application provides such information. Once a part of a sequence is known it is completely trivial to find the rest of the sequence.

With regard to the Examiner's comment about claim 13, applicant notes that claim 13 specifies that the "fragment" must be such

that it can be "used as a primer or as a probe for HA-1 typing." Thus, the Examiner's assertion that the claims do not set forth any functional characteristics that a skilled artisan could use to identify polynucleotides other than by SEQ ID NO. 1 is inaccurate. Finally, applicant has added new claim 20 which remedies the Examiner's concerns, regardless.

Accordingly, applicant respectfully requests reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph.

Rejected under 35 U.S.C. 112, second paragraph

On pages 5-6 of the April 10, 2001 Office Action, The Examiner rejected claims 1-17 under 35 U.S.C. 112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner alleged that claim 1 is indefinite in the recitation of ... anitgen HA-1" as it is unclear if the HA-1 antigen is the HA-1 peptide taught in SEQ ID NO 17, or whether the HA-1 antigen comprises the HA-1 peptide; and that the specification does not define the sequence of the HA-1 antigen, and only discloses that the sequence of the HA-1 peptide "R" allele is disclosed as SEQ ID NO 17.

The Examiner also alleged that claims 2, 4, 5, 8, and 9 are indefinite as these claims refer to figure 5 when referring to a particular nucleic acid sequence, ie: for claim 2, it refers to the "H" and "R" alleles as shown in Fig 5, however figure 5 is composed of two parts, part "a" and part "b", each of which discloses a number of sequences, and Fig 5 is confusing because it assigns SEQ ID NO 17 as a nucleic acid sequence, however SEQ ID NO 17 is an amino acid sequence listing. The Examiner stated that applicant can overcome this rejection by specifying a SEQ

ID NO when referring to a sequence instead of referring to a figure.

The Examiner also alleged that claim 17 is indefinite in the recitation of "...a) possibly, at least one primer" as it is unclear whether a primer of according to claim 10 is present in the kit or not, and the kit of claim 17 is dependent on a method of claim 14, therefore it is unclear if claim 17 should be drawn to a kit or a method.

In response, applicant respectfully submits that claims 1-17, as amended, are in full compliance with 35 U.S.C. § 112, second paragraph. Claims 1-10 and 14-17 are concerned with methods and kits for typing of alleles. These claims are not indefinite. The person skilled in the art is provided with ways to type the named alleles. The prior art readily provides the techniques which a person skilled in the art can use to obtain further sequences of the given alleles. Moreover, the description also demonstrates the significance of the typing method. Very debilitating disease can be prevented from occurring by simply typing these alleles. Thus the person skilled in the art is also given an incentive for finding additional sequences of the named alleles. There is no need, and no legal requirement, for the application to explicitly provide the full sequence of HA-1. The inventors show by example that with the teachings of the invention it is possible to discriminate between them and that it is important to be able to do so. Considering the relative ease with which additional sequences can be obtained along with the teachings of the invention, applicant respectfully requests that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 112, second paragraph.

If a telephone interview would be of assistance in advancing

prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee, other than the enclosed $460.00 extension of time fee, is deemed necessary in connection with the filing of this Response. However, if any additional fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

Gary J. Gershik

Robert D. Katz
Registration No. 30,141
Gary J. Gershik
Registration No. 39,992
Attorneys for Applicants
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231

Gary J. Gershik 10/10/00

Robert D. Katz Date
Reg. No. 30,141
Gary J. Gershik
Reg. No. 39,992

Brief description of the drawings and tables

Table 1

Sequence of the primers used for genomic typing of HA-1 alleles by sequence-specific amplification.

Table 2

Sequence of the primers and probes used for genomic typing of HA-1 alleles by amplification and sequence-specific hybridization.

Table 3

Cellular and genomic typing for HA-1 in three HLA-A*0201 positive families

Table 4.

Comparison of cellular and genomic typing by PCR or LiPA of HA-1 in family 1.

Table 5. ~~(Sequence ID Nos.: 25-32)~~ (Sequence ID Nos.: 25-28)

KIAA0223 sequence polymorphism in mH HA-1 positive and HA-1 negative individuals. Sequencing of HA-1 region in KIAA0223 gene in HA-1 +/+ and HA-1-/- homozygous individuals and KG-1 revealed two alleles differing in two nucleotides resulting in a one amino acid difference (H to R) and designated HA-1^{H} and HA-1^{R} . For DH and vR 6 independent PCR products were sequenced. For KG-1 8 PCR products were sequenced.

Figure 1. Reconstitution of HA-1 with HPLC fractionated peptides eluted from HLA-A2.1 molecules in a ^{51}Cr-release assay with mH HA-1 specific T cell clone 3HA15.

a. Peptides were eluted from 90.10^{9} HA-1 and HLA-A2.1 positive Rp cells and separated using reverse phase HPLC with HFBA as organic modifier.

b. Fraction 24 of the first HPLC dimension that contained HA-1 activity was further fractionated by reverse phase HPLC with TFA as organic modifier.

c. HA-1 containing fraction 27 of the second gradient was further chromatographed with a third shallower gradient consisting of 0.1% acetonitrile/min. Background lysis of T2 by the

The clone Q66.9 is specific for the influenza matrix peptide 58-66. No TNFα production was observed after transfection of the pcDNA3.1(+) vector alone (results not shown).

Figure 4.

a. Binding of HA-1^H and HA-1^R peptides to HLA-A2.1. The binding of HA-1^H and HA-1^R peptides were assayed for their ability to inhibit the binding of fluorescent peptide FLPSDCFPSV to recombinant HLA-A2.1 and β2-microglobulin in a cell free peptide binding assay. One representative experiment is shown. The IC50 is determined on the results of 4 experiments and was 30 nM for VLHDDLLEA and 365 nM for VLRDDLLEA.

b. Reconstitution assay with different concentrations of synthetic HA-1^R peptide with HA-1 specific T cells. The HA-1^R peptide was titrated and preincubated with T2 cells. Three HA-1 specific T cell clones, 5W38, 3HA15 and clone 15 were added and a 4 hr ^{51}Cr-release assay was performed. Background lysis of T2 by the CTL in the absence of any peptide was for 3HA15 4%, for clone 15 10% and for 5W38 2%. Positive control lysis was for 3HA15 46%, for clone 15 47% and 5W38 48%.

Figure 5 ~~(Sequence ID Nos.: 39-42)~~ (Sequence ID Nos.: 17-22)

Sequences and genomic structure of the HA-1 locus. Figure 1a, coding sequences of the H and R alleles of HA-1. Bold characters indicate the polymorphic nucleotides. Figure 1b, exon-intron boundaries of the HA-1 locus. Exon sequences are shown in uppercase, intron sequences in lowercase.

Figure 6

Genomic typing of HA-1 alleles in clinical samples. Genomic typing was performed by sequence-specific amplification, by use of the two primer sets of Table 1. The two upper fragments in the gel originate from the H-allele, the two lower fragments from the R-allele.

Figure 7

HA-1 typing by LiPA of family 1

regression analysis with the prismgraph software. Synthetic peptides were manufactured on a Abimed 422 multiple peptide synthesizer (Abimed, Langenfeld, Germany) and were more than 90% pure as checked by reverse phase HPLC.

1.2.6. RT-PCR amplification and sequencing of KIAA0223 region coding for HA-1

Total or mRNA was prepared from BLCL using the RNAzol methode (Cinna/Biotecx Laboratories, Houston, TX) or according to manufacturer's instructions (QuickPrep mRNA purification Kit, Pharmacia Biotech). CDNA was synthesized with 1 μg RNA as template and with KIAA0223 based reverse primer 5'-GCTCCTGCATGACGCTCTGTCTGCA-3'[1]. To amplify the HA-1 region of KIAA0223 the following primers were used: Forward primer 5'-GACGTCGTCGAGGACATCTCCCAT-3'[2] and reverse primer 5'-GAAGGCCACAGCAATCGTCTCCAGG-3'[3]. Cycle parameters used were denaturation 95 °C, 1 min, annealing 58 °C, 1 min and extension 72 °C, 1 min (25 cycles). The PCR-products were purified using the Magic PCR-Preps DNA purification System (Promega) and direct cloned using the pMosBlue T-vector kit (Amersham LIFE SCIENCE). Six independent colonies from each individual were sequenced using the T7-sequencing kit (Pharmacia Biotech).

1.2.7. HA-1 allele specific PCR amplification

In the case of HA-1 allele specific PCR amplification, cDNA was synthesized as described above. A PCR amplification was performed with allele specific forward primers: for the HA-1^H allele primer H1: 5'-CCT-TGA-GAA-ACT-TAA-GGA-GTG-TGT-GCT-GCA-3'[4], for the HA-1^R allele primer R1: 5'-CCT-TGA-GAA-ACT-TAA-GGA-GTG-TGT-GTT-GCG-3'[5] and for both reaction the reverse primer as described above was used. Cycle parameters used were denaturation 95 °C, 1 min, annealing 67 °C, 1 min and extension 72 °C, 1 min (25 cycles).

1.2.8. Cloning and expression of HA-1^H and HA-1^R allelic region of KIAA0223.

---

1 ~~(Sequence ID No.: 18)~~ (Sequence ID No.: 30)

2 ~~(Sequence ID No.: 19)~~ (Sequence ID No.: 31)

3 ~~(Sequence ID No.: 20)~~ (Sequence ID No.: 32)

4 ~~(Sequence ID No.: 21)~~ (Sequence ID No.: 33)

5 ~~(Sequence ID No.: 22)~~ (Sequence ID No.: 34)

CLAIMS WITH MARKINGS TO SHOW AMENDMENTS

1. (Amended) Method for typing of alleles of the Minor Histocompatibility Antigen HA-1 in a sample ~~with said~~ the method comprising detecting polymorphic nucleotides in the cDNA or genomic nucleic acids of said alleles, thereby typing the alleles.

2. (Amended) Method according to claim 1, further characterized in that said alleles of the Minor Histocompatibility Antigen HA-1 are the ~~H~~ R allele and the ~~R~~ H allele comprising amino acids in sequence as shown in ~~figure 5~~ SEQ ID NOS: 17-20.

3. Method for genomic typing according to claim 1 with said method comprising :
a) contacting the genomic polynucleic acids in the sample with at least one pair of primers, whereby the 5'- and/or the 3'-primer of said at least one pair of primers specifically hybridize to target regions comprising polymorphic nucleotides in said alleles, and performing an amplification reaction;
b) for each of said at least one pair of primers detecting whether or not in step a) an amplification product is formed;
c) inferring from the result of step b) which HA-1 allele is present in said sample.

4. (Amended) Method according to claim 1, further characterized in that:
said at least one pair of primers comprises a 5'-primer that specifically hybridizes to a target region comprising the nucleotides at position 4 or at positions 4 and 8 in the HA-1 allele, or, said at least one pair of primers comprises a 3'-primer that specifically hybridizes to a target region comprising the nucleotides at position 8 or at positions 4 and 8 in the HA-1 allele, with said positions being indicated in ~~figure 5~~ SEQ ID NOS: 17-20.

5. (Amended) Method according to claim 4, further characterized in that:
said 5'-primer is combined with a 3'-primer specifically hybridizing to a target region in intron a, and/or said 3'-primer is combined with a 5'-primer specifically hybridizing to a target region in exon a, with intron a and exon a being indicated in ~~figure 5~~ SEQ ID NO: 21-22.

6. Method according to claim 1 further characterized in that the primers are chosen from the following

list:
SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7.

7. Method for genomic typing according to claim 1 with said method comprising:
a) amplifying a fragment of said alleles, with said fragment comprising at least one polymorphic nucleotide, by use of at least one pair of primers specifically hybridizing to conserved target regions in said alleles;
b) hybridizing the amplified product of step a) to at least one probe specifically hybridizing to a target region comprising one or more polymorphic nucleotides in said allele;
c) inferring from the result of step b) which HA-1 allele is present in said sample.

8. (Amended) Method according to claim 7, further characterized in that said at least one pair of primers comprises a 5'-primer specifically hybridizing to a conserved target region in exon a and/or a 3'-primer specifically hybridizing to a conserved target region in intron a, with exon a and intron a being indicated in ~~figure 5~~ SEQ ID NOS: 21-22.

9. (Amended) Method according to claim 7 further characterized in that said at least one probe specifically hybridizes to a target region comprising the nucleotides at position 4 and/or 8 in the HA-1 allele, with said positions being indicated in ~~figure 5~~ SEQ ID NOS: 17-20.

10. Method according to claim 7 further characterized in that:
said primers are chosen from the following list:
SEQ ID NO 2, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, and/or said probes are chosen from the following list:
SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 16.

11. A primer for use in a method according to claim 1 for genomic typing of alleles of the Minor Histocompatibility Antigen HA-1.

12. A probe for use in a method according to claim 7 for genomic typing of alleles of the Minor Histocompatibility Antigen HA-1.

13. An isolated polynucleic acid identified by SEQ ID NO 1, or SEQ ID NO 17 or SEQ ID NO 18 or an isolated polynucleic acid displaying at least 80% sequence homology to said polynucleic acids, or any fragment of said polynucleic acids that can be used as a primer or as a probe for HA-1 typing.

14. A method for genomic typing of alleles of the Minor Histocompatibility Antigen HA-1 according to claim 1 by means of sequencing said allele.

15. A diagnostic kit for typing of alleles of the Minor Histocompatibility Antigen HA-1 according to claim 3 with said kit comprising:
a) at least one primer according to claim 1.
b) optionally, an enzyme and/or reagents enabling the amplification reaction;
c) optionally, means enabling detection of the amplified products.

16. A diagnostic kit for genomic typing of alleles of the Minor Histocompatibility Antigen HA-1 according to claim 7 with said kit comprising:
a) at least one primer according to claim 10.
b) at least one probe according to claim 10.
c) optionally, an enzyme and/or reagents enabling the amplification reaction, and/or reagents enabling the hybridization reaction.

17. (Amended) A diagnostic kit for genomic typing of alleles of the Minor Histocompatibility Antigen HA-1 according to claim 14, with said kit comprising:
a) ~~possibly,~~ at least one primer according to claim 10;
b) optionally, an enzyme and/or reagents enabling the amplification reaction, and/or reagents enabling the sequencing reaction.

18. (Amended) A method for typing HA-1 alleles comprising using antibodies specifically detecting the HA-1 alleles as shown in ~~figure 5~~ SEQ ID NOS: 17-20.

19. (Amended) A diagnostic kit for typing HA-1 alleles comprising antibodies specifically detecting the HA-1 alleles as shown in ~~figure 5~~ SEQ ID NOS: 17-20.

20. (New) An isolated polynucleic acid identified by SEQ ID NO. 1, or SEQ ID NO. 17, or SEQ ID NO. 18 or an isolated polynucleic acid displaying at least 80% sequence homology to said polynucleic acid.